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Diaha-Kouamé Amenan Claude Aimée
Laboratoire d'Endocrinologie et
Biologie de la Reproduction,
Université Félix Houphouët-
Boigny, Abidjan, Côte d'Ivoire,
West Africa

Yao Kouassi Patrick
Laboratoire de Zoologie et
Biologie Animale UFR de
Parasitologie et Ecologie
Parasitaire, Université Félix
Houphouët-Boigny, Côte
d'Ivoire, West Africa

Tano Djè Kévin Christian
Université Jean Lorougnon
Guédé de Daloa, UFR
Agroforesterie, Côte d'Ivoire,
West Africa

Azokou Alain
Centre Suisse de Recherches
Scientifiques en Côte d'Ivoire,
West Africa

Kouakou Koffi
Laboratoire d'Endocrinologie et
Biologie de la Reproduction,
Université Félix Houphouët-
Boigny, Abidjan, Côte d'Ivoire,
West Africa

Correspondence

Diaha-Kouamé Amenan Claude Aimée
Laboratoire d'Endocrinologie et
Biologie de la Reproduction,
Université Félix Houphouët-
Boigny, Abidjan, Côte d'Ivoire,
West Africa

Evaluation of acaricide activity in the leaves extracts of four medicinal local plants on *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888)

Diaha-Kouamé Amenan Claude Aimée, Yao Kouassi Patrick, Tano Djè Kévin Christian, Azokou Alain and Kouakou Koffi

Abstract

The evolution of the resistance of the tick *Rhipicephalus (Boophilus) microplus* to synthetic acaricides gave rise to the need for new scientific research on other ways to control this tick. In this regard, various plant studies have been developed in an attempt to find extracts with acaricidal properties. Aqueous extracts and ethanolic extracts of leaves of *Azadirachta indica* (Meliaceae), *Ricinus communis* (Euphorbiaceae), *Cymbopogon citratus* (Poaceae) and *Tithonia diversifolia* (Asteraceae) were contacted at different concentrations with *R. (B.) microplus*. Tests on larvae and engorged females were carried out at concentrations ranging from 25 to 400 mg/ml in the laboratory of the Swiss scientific research center in Adiopodoumé, Côte d'Ivoire. The treatments and controls were carried out in three replicates. At the concentrations of 200 and 400 mg/ml, the ethanolic extracts of these 4 plants were all very active with a mortality rate of 100% of the larvae. The ethanolic extract of *C. citratus* resulted in the highest mortality at different concentrations with a lethal concentration (LC₅₀) of 35.924 mg/ml. On the engorged females, the aqueous extract of *C. citratus* induced the strongest inhibition of laying, 58.20% at the concentration of 400 mg/ml. At this same concentration, the extract of *R. communis* was more active on the hatch with a low hatching rate of eggs (25.06%). The application of these plant extracts showed acaricidal activity against *R. (B.) microplus*, so it is obvious that the use of plant extracts for the control of this tick is a possible alternative.

Keywords: Resistance, Plant extracts, *Rhipicephalus (Boophilus) microplus*, Mortality rate

1. Introduction

Ticks and transmitted diseases cause many problems for the cattle industry worldwide [1]. About 80% of cattle worldwide are tick-infected. In this respect, they constitute a brake on livestock development in several countries and a source of loss and poverty for small-scale farmers [2]. The main control method involves the use of synthetic acaricides. However, the development of resistant strains of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) in different parts of the world has rendered several chemical agents ineffective [3]. In addition, pollution and contamination of meat and milk are associated with this type of control [4]. This species is arguably the ectoparasite of cattle most economically important in the tropical and subtropical regions of the world.

Since the introduction of *R. (B.) microplus* in Côte d'Ivoire and several other regions of West Africa [5], breeders complain of heavy infestations of their animals by this tick that seems to resist all the chemicals they have on the market [6]. This poses a serious threat to livestock in West Africa because of the invasiveness of this tick and, above all, its ability to compete and establish itself at the expense of other native species of ticks such as *Rhipicephalus (Boophilus) annulatus*, *Rhipicephalus (Boophilus) geigy* or *Rhipicephalus (Boophilus) decoloratus* [7]. It is currently one of the most resistant ticks to acaricides in infested farms. In order to cope with this, new alternative methods of control are being explored, including the use of plant extracts with acaricidal properties [8]. Indeed, plant extracts are generally of low toxicity to mammals, soluble in water, have few side effects and are rarely or not at the origin of resistances within the tick populations [9].

The present study evaluates the larvae and engorged females of *R. (B.) microplus*, the efficacy of the ethanolic and aqueous extracts of four plants: *Tithonia diversifolia*, *Azadirachta indica*, *Ricinus communis*, *Cymbopogon citratus*.

2. Materials and Methods

2.1 Collection of plant and powder conditioning

The leaves of four (4) plants were harvested in the municipality of Abidjan. These include *Tithonia diversifolia* (Asteraceae), *Azadirachta indica* (Meliaceae), *Ricinus communis* (Euphorbiaceae), *Cymbopogon citratus* (Poaceae). These organs were dried and then ground with a mixer, until a powder is obtained and then packaged in bottles and stored at 18 °C.

2.2 Preparation of different concentrations of plant extracts and the control solution

The laboratory work was carried out in one of the laboratories of the Centre Suisse de Recherches Scientifiques (CSRS) in Côte d'Ivoire. For each plant, 30 g of powder were mixed in 300 ml of 95% ethanol for the ethanol extracts and in 300 ml of distilled water for the aqueous extracts. The ethanolic and aqueous extracts were prepared by mixing 10 times more solvent than vegetable powder, with a mechanical stirrer for 24 hours. These mixtures were filtered and the concentrated filtrates were brought to an oven at 35 °C for 4 days to complete the evaporation. These ethanolic and aqueous vegetable extracts were used to test respectively the sensitivity of larvae and engorged females of *R. (B.) microplus*. With regard to the preparation of the concentrations to be tested, the extracts were diluted in cascade, going from the stock solution to the less concentrated. The control solution was used to prepare a series of five solutions of concentration 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml for each of the extracts of the four plants. For each concentration, three repetitions were performed.

2.3 Collection of ticks

The *R. (B.) microplus* engorged females came from a cattle farm located in Bingerville. They were harvested alive on zebu animals and brought back to the laboratory of the Centre Suisse de Recherches Scientifiques (CSRS) in Adiopodoumé (suburbs of Abidjan) for identification, and then distributed in batches of 5 to 10 in dry bottles filled with very fine mesh. They were then placed in an oven at a temperature of 27 ± 1 °C and 85 to 90% relative humidity for their laying. At the end of the egg laying, the dead females were removed from the flasks to isolate the eggs before incubation. The incubation of the eggs took place under the same conditions as the laying of the eggs; it is the same for the conservation of the newly emerged larvae. As far as the control solution is concerned, an olive oil and trichlorethylene mixture of two volumes of trichlorethylene for one volume of olive oil has been prepared for this purpose.

2.4.2. Adult Immersion Test (AIT)

The use of the Adult Immersion Test (AIT) was used to test the efficacy of aqueous leaves extracts of the four (4) plants targeted on the engorged females *R. (B.) microplus*. The same tick strain used for LPT was used for this test, with the difference that efficacy was evaluated for gorged females weighing between 150 and 350 mg and larger than 4.5 mm^[11]. These engorged females were immediately taken to the CSRS laboratory for testing (Figure 2). A sieve was used to separate the ticks below the size and weight indicated. The ticks were

2.4 Sensitivity tests

2.4.1 Larval Packet Test (LPT)

The Larval Packet Test (LPT) was used to determine the acaricidal effect of the ethanolic extracts of the leaves of four (4) plant species on the larvae of *R. (B.) microplus*. Concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml of each extract of the four plants were evaluated at the rate of three replicates per plant extract. For this purpose, pockets of filter paper whatmann N°1 (7.5cm × 8.5cm) were made, with 3 pockets per concentration and 1 control pocket for each ethanol extract. Thus, in a pipette, 0.67 ml of each solution was taken to impregnate each of the corresponding pockets which will then be for two hours under the hood. The four control pockets received the same volume of control solution. The lateral edges of each of the pockets were sealed with two clasps (Figure 1). Approximately 100 to 150 larvae of 14 to 21 days were gently transferred to each pouch using a brush.



Fig 1: Pouch after deposition of larvae

For each plant extract, average larval mortality rates were calculated and corrected by Abbott^[10] formula recommended by FAO^[3].

$$\text{Mortality}(\%) = \frac{\text{Dead larvae}}{\text{Total larvae}} \times 100$$

$$\text{Average mortality}(\%) = \frac{\text{Mortality1} + \text{Mortality2} + \text{Mortality3}}{3} \times 100$$

$$\% \text{ Mortality (corrected)} = \frac{\% \text{ mortality (test group)} - \% \text{ mortality (control group)}}{100 - \% \text{ mortality (control group)}} \times 100$$

then washed and then dried on towel paper. In boxes, the engorged females were distributed due to 10 females which were constituted by box and then weighed. To dilute the aqueous extracts of the four plants, we used distilled water to obtain a final volume of 10 ml with the following concentrations: 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml. For each concentration, three repetitions were performed.



Fig 2: engorged females of *R. (B.) microplus*

For each control solution and each of the acaricidal test solutions and starting with the lowest concentration, each group of ticks was placed directly into a container containing 10 ml of the treatment solution. The whole was stirred vigorously and left for 5 minutes. After this latency, the gorged ticks were removed from the mixture and then placed on a clean paper towel for drying. After drying, all ticks in each group are placed in a dry box clogged with a very fine mesh and labeled. The ticks are placed in an oven at a temperature of 27-28 °C and a relative humidity of between

80-95%. The number of spawning females was recorded and the count of dead ticks was recorded daily.

After 18 days of egg laying, ticks are removed from their boxes and the eggs laid are weighed and then returned to the oven under the same conditions as the laying of eggs for the future estimation of outbreaks. At the end of the hatch (between 20 and 25 days) the hatching rate of the eggs was estimated. This estimate is visual and three replications of this estimate have been performed. The effect of the extracts was evaluated by determining the mortality of the females and then the egg laying and hatching, while comparing the treated females to the untreated.

The efficacy of the laying was obtained according to the formulas:

$$IE(\text{index of egg laying}) = \frac{\text{weight of eggs laid (g)}}{\text{weight of females (g)}}$$

$$\text{Egg laying inhibition (\%)} = \frac{IE(\text{control}) - IE(\text{treated group})}{IE(\text{control})} \times 100$$

At the end of the egg hatch, the hatching rate of the eggs was estimated. The efficacy of the plant extract is determined by comparing the estimated reproduction (REI) of each group of treated ticks with that of the control ticks, as proposed by Drummond *et al.* [11] through the following formulas:

$$REI(\text{reproductive efficacy index}) = \frac{\text{weight of eggs (g)} \times \% \text{ of hatching}}{\text{weight of female (g)}} \times 20000$$

20 000 = number of larvae at approximately 1.0 g of eggs (number experimentally obtained)

$$EP(\text{efficacy of the extracted product}) = \frac{REI(\text{control group}) - REI(\text{treated group})}{REI(\text{control group})} \times 100$$

2.5 Statistical analysis

The data collected during the biological tests were entered with Microsoft Office Excel version 2013. Data processing was carried out using the software Statistica version 9.6. An analysis of variance (ANOVA) revealed the significant differences between the larval mortality rates of the different extracts. The Fisher, Student, Kruskal-Wallis tests (at the 5% threshold) and correlation tests were used. LC_{50} is calculated by Probit analysis based on mortalities obtained after 24 hours at different concentrations. The effect of plant extracts on the reproductive parameters of the fed-up female ticks and the hatching rate of the larvae was determined using the formulas of Drummond *et al.* [11].

3. Results

3.1 Acaricidal efficacy of ethanol extracts on larvae

3.1.1 Variation in larval mortality rate

The ethanolic extracts of the four plants had varying effects on the larvae of *R. (B.) microplus*, at the different concentrations tested (Figure 3). At concentrations of 200 and 400 mg/ml, the ethanolic extracts showed an acaricidal effect with a mortality rate of 100%. At these concentrations these plant species are very active.

At the concentration of 100 mg/ml it was only the extract of *C. citratus* which was very active with a mortality rate of 100%. The other three extracts *A. indica*, *R. communis* and *T. diversifolia* were active at the same concentration with mortality rates respectively of 75; 78 and 77%. At the lowest concentrations, *C. citratus* showed the highest mortality rates with 80% mortality at the 50 mg/ml concentration and 71% mortality at the 25 mg/ml concentration. For each concentration, the highest larval mortality rates were obtained with the *C. citratus* extract. This extract showed a higher larval mortality rate than the other three plants and follows in decreasing order *R. communis*, *T. diversifolia* and *A. indica*. For all of these extracts, dose-response effects were observed. The number of dead larvae increases with the concentration (400 to 25 mg/ml).

Statistical analyzes using the Fisher test on mean deaths for the four extracts are significant. The P-Value for the extracts of *A. indica*, *R. communis*, *C. citratus*, and *T. diversifolia* are respectively (0.00085) (0.05269) (0.0137) (0.0023). The comparison of the two-to-one averages through the student test shows that the averages are different from one another. Correlation tests show that the toxicities of the extracts have a strong positive correlation with the concentrations.

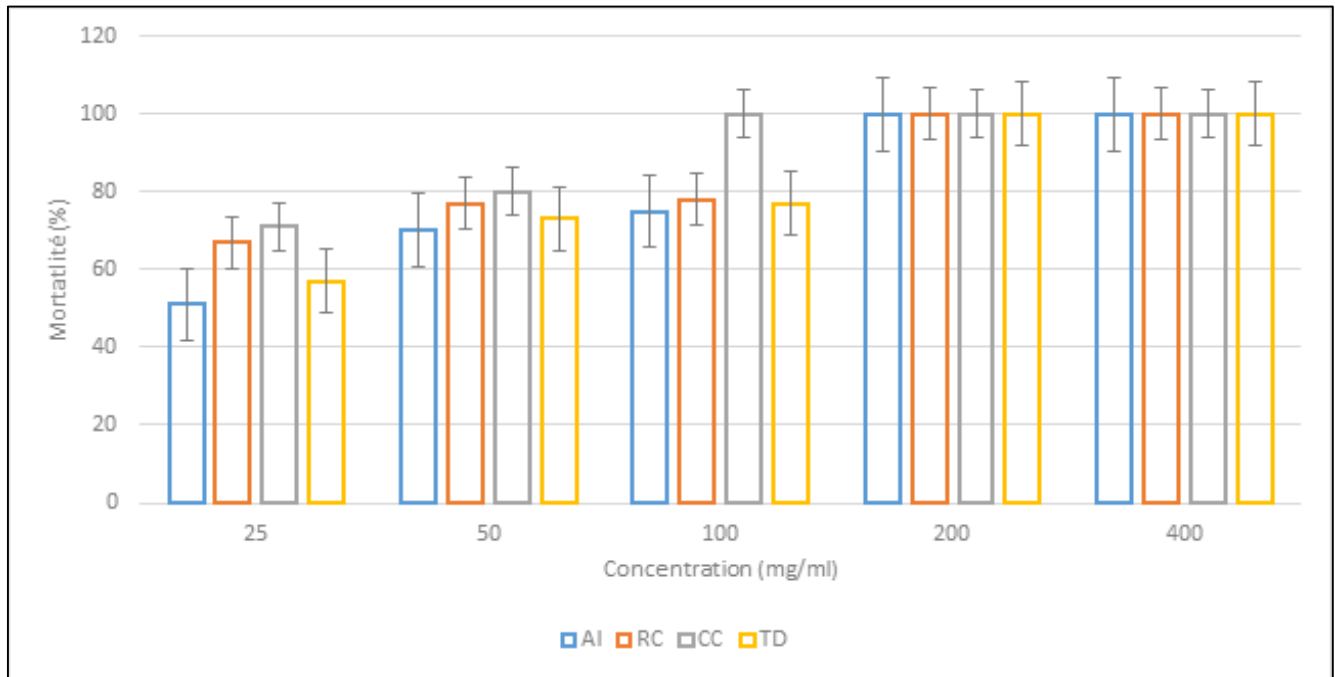


Fig 3: Larval mortality as a function of the concentration of ethanolic extracts in plants. AI: *Azadirachta indica*; RC: *Ricinus communis*; CC: *Cymbopogon citratus*; TD: *Tithonia diversifolia*

3.1.2 Lethal Concentrations LC₅₀

Three groups of extracts classified from the most effective to at least active according to their toxicity (LC₅₀), on *R. (B.) microplus*, are distinguished (Table 1). Group 1 contains the ethanolic extract of *C. citratus*. This extract has a higher toxicity than all the other ethanolic extracts. In Group 2, extracts of *R. communis* and *T. diversifolia* are found. On the other hand, the *A. indica* extract is the least toxic.

The values of the LC₅₀ calculated for *R. (B.) microplus* larvae showed that among the 4 extracts tested, *C. citratus* proved to be more interesting in terms of toxicity. The lowest LC₅₀ for larvae was observed for the *C. citratus* extract, which had a higher acaricidal effect for larval control of *R. microplus* with an LC₅₀ of 35.924 mg/ml. The three other plants also showed a very interesting acaricidal efficacy with LC₅₀ of: 54.263 mg/ml (*R. communis*), 55.691 mg/ml (*T. diversifolia*) and 62.903 mg/ml (*A. indica*).

Table 1: Rank Order of Decreasing Lethal Concentration of Ethanol Extracts

Order of efficacy	Extracts	LC ₅₀ (mg/ml)
1	<i>Cymbopogon citratus</i> (Poaceae)	35,924 ± 0 a
2	<i>Ricinus communis</i> (Euphorbiaceae)	54,263 ± 0,1 b
3	<i>Tithonia diversifolia</i> (Asteraceae)	55,691 ± 0 b
4	<i>Azadirachta indica</i> (Meliaceae)	62,903 ± 0 c

Within the same column, the values followed by the same letters are not significantly different at the 5% threshold according to the Kruskal-Wallis test ($p < 0.05$; Anova - analysis of variance). LC₅₀: Lethal Concentration 50, a quantitative indicator of the toxicity of a substance.

3.2 Acaricidal efficacy of aqueous extracts on engorged females

3.2.1 Acaricide effect on females of *R. (B.) microplus*

Mortality of engorged females was observed prior to laying. Thus, at the end of the laying, of the 640 gorged females, we observed a total of 5 dead females, a mortality rate of 0.78%. The mortality of engorged females was therefore very low.

3.2.2 Effect of extracts on laying

The females *R. (B.) microplus* weighing 2.31 to 2.49 g laid eggs weighing between 0.38 g (females treated with 400

mg/ml of *C. citratus*) and 0.91 g (control females). The spawning efficiency indices of engorged females varied from 0.16 (females treated with 400 mg/ml of *C. citratus*) to 0.38 (control females). At the highest concentration of 400 mg/ml, the inhibition levels of the 4 extracts were greater than 50%. At this same concentration, *C. citratus* had the strongest laying inhibition of 58.20%. For the other concentrations (100; 50 and 25 mg/ml), *T. diversifolia* had the highest inhibition effects of 35.68; 34.38 and 24.22% (Table 2). Correlation tests show that the weights of eggs laid have a strong negative correlation with the concentrations.

Table 2: Inhibition of the laying of the engorged females of *R. (B.) microplus* treated with 4 aqueous extracts of plants

Product	Concentration (mg/ml)	Females weight (g)	Egg mass weight (g)	IEP (index of egg laying)	Egg laying inhibition (%)
<i>Azadirachta indica</i> (Meliaceae)	Control	2.43 ± 0.01	0.92 ± 0.01	0.38	
	25	2.41 ± 0.04	0.75 ± 0.00	0.31	18.11
	50	2.43 ± 0.01	0.72 ± 0.01	0.30	21.61
	100	2.46 ± 0.00	0.65 ± 0.02	0.27	30.07
	200	2.41 ± 0.01	0.65 ± 0.00	0.27	28.51
	400	2.47 ± 0.02	0.44 ± 0.04	0.18	52.79
<i>Ricinus communis</i> (Euphorbiaceae)	Control	2.31 ± 0.02	0.90 ± 0.05	0.39	
	25	2.36 ± 0.01	0.88 ± 0.03	0.37	4.30
	50	2.31 ± 0.02	0.83 ± 0.00	0.36	7.46
	100	2.38 ± 0.05	0.74 ± 0.01	0.31	19.91
	200	2.33 ± 0.03	0.64 ± 0.02	0.27	29.56
	400	2.31 ± 0.01	0.42 ± 0.03	0.18	53.79
<i>Cymbopogon citratus</i> (Poaceae)	Control	2.39 ± 0.02	0.91 ± 0.01	0.38	
	25	2.34 ± 0.00	0.68 ± 0.00	0.29	23.37
	50	2.31 ± 0.03	0.67 ± 0.02	0.29	23.28
	100	2.38 ± 0.00	0.61 ± 0.01	0.26	32.50
	200	2.32 ± 0.01	0.52 ± 0.01	0.22	41.63
	400	2.39 ± 0.02	0.38 ± 0.00	0.16	58.20
<i>Tithonia diversifolia</i> (Asteraceae)	Control	2.49 ± 0.02	0.95 ± 0.01	0.38	
	25	2.46 ± 0.01	0.71 ± 0.02	0.29	24.22
	50	2.43 ± 0.0	0.61 ± 0.01	0.25	34.38
	100	2.41 ± 0.03	0.59 ± 0.03	0.25	35.68
	200	2.48 ± 0.01	0.58 ± 0.01	0.23	38.66
	400	2.45 ± 0.01	0.45 ± 0.04	0.18	51.70

3.2.3 Effects of extracts on hatching of eggs laid by treated engorged females

The hatching rates of eggs laid by treated engorged females varied from 25.06% (females treated with 400 mg/ml of *R. communis*) to 77.81% (females treated with 25 mg/ml of *T. diversifolia*). In *R. communis* extract at 400 mg/ml resulted in the greatest reduction in the outbreak with 87.93% efficacy

and a reproductive efficacy index of 9. For other concentrations 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, the highest efficacy were recorded respectively with the aqueous extract of *C. citratus* 70.46; *T. diversifolia* 65.79; *A. indica* 57.70 and *C. citratus* 39.78% (Table 3).

Correlation tests show that the weights of hatched eggs have a strong negative correlation with concentrations.

Table 3: Effects of 4 aqueous plant extracts on egg laying, hatching of eggs spawned by female rips of *R. (B.) microplus* treated at different concentrations

Product	Concentration (mg/ml)	Females weight (g)	Egg mass weight (g)	Weight of hatched eggs (g)	% of hatching	REI (reproductive efficacy index)	EP (efficacy of the extracted product)
<i>Azadirachta indica</i> (Meliaceae)	Control	2.43 ± 0.01	0.92 ± 0.01	0.88 ± 0.03	95.22	72.18	
	25	2.41 ± 0.04	0.75 ± 0.00	0.58 ± 0.01	77.14	47.88	33.67
	50	2.43 ± 0.01	0.72 ± 0.01	0.37 ± 0.01	51.39	30.53	57.70
	100	2.46 ± 0.00	0.65 ± 0.02	0.31 ± 0.02	46.93	24.88	65.53
	200	2.41 ± 0.01	0.65 ± 0.00	0.32 ± 0.01	48.55	26.31	63.55
	400	2.47 ± 0.02	0.44 ± 0.04	0.21 ± 0.02	46.61	16.68	76.89
<i>Ricinus communis</i> (Euphorbiaceae)	Control	2.31 ± 0.02	0.90 ± 0.05	0.86 ± 0.03	95.88	74.54	
	25	2.36 ± 0.01	0.88 ± 0.03	0.66 ± 0.02	75.06	55.85	25.07
	50	2.31 ± 0.02	0.83 ± 0.00	0.62 ± 0.01	74.97	53.94	27.64
	100	2.38 ± 0.05	0.74 ± 0.01	0.37 ± 0.04	49.53	30.84	58.63
	200	2.33 ± 0.03	0.64 ± 0.02	0.32 ± 0.02	49.37	27.04	63.72
	400	2.31 ± 0.01	0.42 ± 0.03	0.10 ± 0.03	25.06	9	87.93
<i>Cymbopogon citratus</i> (Poaceae)	Control	2.39 ± 0.02	0.91 ± 0.01	0.87 ± 0.01	95.16	72.38	
	25	2.34 ± 0.00	0.68 ± 0.00	0.51 ± 0.03	74.78	43.59	39.78
	50	2.31 ± 0.03	0.67 ± 0.02	0.50 ± 0.02	74.48	43.46	39.96
	100	2.38 ± 0.00	0.61 ± 0.01	0.31 ± 0.03	50.08	25.71	64.48
	200	2.32 ± 0.01	0.52 ± 0.01	0.25 ± 0.02	48.16	21.38	70.46
	400	2.39 ± 0.02	0.38 ± 0.00	0.18 ± 0.04	47.37	15.06	79.19
<i>Tithonia diversifolia</i> (Asteraceae)	Control	2.49 ± 0.02	0.95 ± 0.01	0.90 ± 0.04	94.95	72.53	
	25	2.46 ± 0.01	0.71 ± 0.02	0.55 ± 0.01	77.81	45.04	37.90
	50	2.43 ± 0.0	0.61 ± 0.01	0.43 ± 0.03	70.11	35.14	51.55
	100	2.41 ± 0.03	0.59 ± 0.03	0.30 ± 0.02	50.51	24.81	65.79
	200	2.48 ± 0.01	0.58 ± 0.01	0.30 ± 0.01	50.77	23.79	67.20
	400	2.45 ± 0.01	0.45 ± 0.04	0.22 ± 0.05	47.79	17.63	75.69

4. Discussion

In our experiment, the various leaves extracts studied were subjected to biological tests against the *R. (B.) microplus* species in the laboratory. After 24 hours of contact, the mortality rate varied according to the concentrations and the ethanolic extracts gave satisfactory results insofar as they showed a large larvicidal effect. Thus, in this work we explored the potential of the bioactive compounds of *T. diversifolia*, *A. indica*, *R. communis* and *C. citratus* for control of *R. (B.) microplus*. The ethanolic and aqueous extracts of the leaves of these plants showed toxic effects respectively on larvae and on the engorged females of *R. (B.) microplus*. Thus, we studied the acaricidal activity of these ethanolic extracts on the larvae of age between 14 and 21 days. Toxicity tests revealed after 24 hours of exposure to larvae LC₅₀ of 35.924 mg/ml for *C. citratus* of 54.263 mg/ml for *R. communis* of 55.691 mg/ml for *T. diversifolia* of 62.903 mg/ml for *A. indica*. Thus *C. citratus* had a higher acaricidal activity for the control of *R. (B.) microplus* larvae. Furthermore, the use of *Cymbopogon winterianus* Jowitt essential oil showed a LC₅₀ of 41 mg/ml on larvae [12]. Also the application of the essential oils of *Cymbopogon. Martinii* and *Cymbopogon schoenanthus* in the larval test gave LC₅₀ and CL90 respectively for these plants 4.7 mg/ml and 6.3 mg/ml, 5.7 mg/ml and 9.6 mg/ml [13]. *A. indica* is an indigenous plant commonly cultivated mainly in India and experiments with this plant by other authors have shown some acaricidal properties of its extracts [14]. In addition to *A. indica* several studies have been conducted on other plants, thus, Pathak *et al.*, [15] evaluated the acaricidal effect leaves and bark extract of *A. indica*, leaves of two (2) plants (*Vitex nungundo*, *Acorus calamus*) and the rhizome of *Pongamia pinnata*, on Ixodid ticks of small ruminants. Kumar *et al.* [16] revealed the *in vitro* adulticidal activity of the raw methanolic extract of *Thevetia nereifolia*. As in our study, many other studies have reported results of *in vitro* efficacy of plant species on *R. (B.) microplus* better or similar to the results of commercial acaricides. In the case of *Piper aduncum*, the results showed an activity similar to those of various synthetic acaricides which today do not reach 90% efficiency due to resistance problems of this species [17].

In the present study, the toxic effects of the aqueous extracts of these four plants at different concentrations were also evaluated on the females. Mortality of females prior to laying was low (0.78%). These plant extracts would therefore not have an adulticidal effect on *R. (B.) microplus* and could act as growth inhibitors as is the case with Fluzuron [18]. Indeed, this chemical molecule has no adulticidal effect on the fed females of the *R. (B.) microplus* tick [19]. On the other hand Fluzuron interrupts the life cycle of the tick at different points interfering with the formation of the cuticle. Thus, this molecule would regulate the growth of the tick by inhibiting the incorporation of chitin into the cuticle of the tick [20]. This results in the death of larvae and nymphs because they cannot move on to the next stage and prevent adult females from producing viable eggs and also affect the egg hatching of the target parasite [21]. As regards the egg-laying inhibition, *C. citratus* had a strong inhibition of laying at the highest concentrations (400 mg/ml, 200 mg/ml), which were respectively 58.2 and 41.63. For the other concentrations (100 mg/ml, 200 mg/ml and 25 mg/ml), *T. diversifolia* had the highest inhibition effects of 35.68; 34.38 and 24.22. In terms of reproductive efficacy, we obtained the best results with *R. communis* extract at the concentration of 400 mg/ml, with a

value of 87.93% for very low efficacy of the reproduction which is 9. The best results of the efficacy of the product for the other concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml) are respectively *C. citratus* 70.46%; *T. diversifolia* 65.79%, *A. indica* 57.70% and *A. indica* 39.78%. Studies on the reproduction of engorged females *R. (B.) microplus* with *A. indica* leaves extracts have shown that this plant inhibits the production of *R. (B.) microplus* tick eggs [22] and weekly spraying with neem seed extracts decreased the number of ticks on cattle [23] and goats [24] in southern Africa. Low interference with reproductive efficacy (32%) was obtained by Costa *et al.* [25] using an aqueous alcoholic extract of *A. indica* leaves at a concentration of 200 mg/ml. In our study, at the same concentration, our results are better, we obtained a coefficient of reproductive efficacy 26.31. Also in this study the ethanolic and aqueous extracts of *T. diversifolia* showed an acaricidal property on larvae and females of *R. (B.) microplus*. In addition, the acaricidal activity on *R. (B.) microplus* has not been previously reported. Moreover, this plant is known for its insecticidal effects against termites in rice cultivation and many other crops [26]. Various species of the genus *Cymbopogon* (Poaceae) were also tested against *R. (B.) microplus*. The essential oil of *Cymbopogon winterianus* Jowitt was tested against larvae and engorged females and showed total inhibition of egg laying at a concentration of 100 mg/ml and inhibition of hatching at a concentration of 71.4 Mg/ml [12]. The essential oil of *C. nardus* caused 79% inhibition of reproduction at a concentration of 10 mg/ml [27]. On the other hand, Costa *et al.* [25] found that this plant had a low efficacy (17%) against females using a hydroalcoholic leaf extract at a concentration of 200 mg/ml. The lemongrass leaf extract at a concentration of 20 mg / ml had an efficacy of 18.35% against gorged females [28]. The alcoholic extract of *C. citratus* gave a partial control of the tick *in vitro*, with 45% efficiency at a concentration of 230 mg/ml [29]. Silva *et al.* [30], with the same extract obtained 42% product efficacy (PE) on the engorged females of *R. (B.) microplus* at a concentration of 100 mg/ml. In our work at this same concentration we obtained an efficiency 64.48%. Moreover, the work of Ghosh *et al.* [31] reported that leaves extract of *R. communis* proved to be considerably effective against *R. (B.) microplus* ticks resistant to organophosphates and pyrethroids. Like our authors, our study demonstrated the acaricidal activity of leaves of *T. diversifolia*, *A. indica*, *R. communis* and *C. citratus* and found that *C. citratus* and *R. communis* exhibited the highest efficiencies on larvae and engorged females. For these four plants, as regards the larvicidal action and the inhibiting actions of egg laying and hatching, the efficacy of the extracts increases with the dose used. Also Tahiri, [32] showed that the extract of Kinkeliba, *Combretum micranthum* (Combretaceae), increases with the dose used on termites. On the other hand, the efficacy of alcoholic extracts of carica papaya seeds and the hexane extract of papaya pulp (Caricaceae) have their optimal low-dose actions against termites [33].

5. Conclusion

Our results show that, after 24 hours of exposure, the ethanolic extracts of *A. indica*, *R. communis*, *C. citratus* and *T. diversifolia* showed good efficacy against the larvae of *R. (B.) microplus*. However, *C. citratus* had a higher larvicidal activity for control of *R. (B.) microplus* with an LC₅₀ of 35.924 mg/ml. On adult ticks, *C. citratus* also showed relatively high levels of laying inhibition of 58.2% and

41.63% at the highest concentrations of 400 mg/ml and 200 mg/ml, respectively. The adulticidal efficacy is given by a very low mortality rate (0.78%) as these plants acted as growth inhibitors.

For the efficacy of the products on the egg laying we obtained the best results with the extract of *C. citratus* at the highest concentration and for the other concentrations the best results were obtained with the extract of *T. diversifolia*. With respect to the egg hatching effect, at the highest concentration, *R. communis* gave the best results (87.93%). The best results of the efficacy of the extracts for the other concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml) are respectively *C. citratus* 70.46%; *T. diversifolia* 65.79%, *A. indica* 57.70% and *A. indica* 39.78%. In the context of tick control, the extracts of these plants can be used according to their effects on the different stages of development of the tick *R. (B.) microplus*. Thus, it would be possible to produce solutions based on these plants in order to effectively combat this tick and to avoid the cases of resistance associated with the usual acaricides.

6. References

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